PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

(Attorney Docket No. SIR-MIS-00001-US-CIP[4])

IN THE APPLICATION OF:			
McSwiggen *et al.*			
Serial No.:	10/757,803	Examiner:	BOWMAN, Amy Hudson
Filed:	January 14, 2004	Group Art Unit:	1635
Title	RNA Interference Mediated Inhibition of Gene Expression Using Chemically Modified Short Interfering Nucleic Acid (siNA)	Confirmation No.:	5421

BRIEF ON APPEAL

Peter Haeberli
Attorney for Applicant
Merck
1700 Owens Street, 4th Floor
San Francisco, CA 94158

Phone: (415) 814-8491
Fax: (415) 512-7022

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

(Attorney Docket No. SIR-MIS-00001-US-CIP[4])

IN THE APPLICATION OF:			
McSwiggen *et al.*			
Serial No.:	10/757,803	Examiner:	BOWMAN, Amy Hudson
Filed:	January 14, 2004	Group Art Unit:	1635
Title	RNA Interference Mediated Inhibition of Gene Expression Using Chemically Modified Short Interfering Nucleic Acid (siNA)	Confirmation No.:	5421

BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal from the Final Rejection mailed October 21, 2010, and the Advisory Action mailed December 30, 2010 and re-mailed January 24, 2011. This brief is submitted along with the large entity fee of $540. A notice of appeal was timely filed January 14, 2011. No additional fee is believed to be due. In the event of any variance between the amounts enclosed and the Patent and Trademark Office charges, the Commissioner is authorized to charge or credit any difference to our Deposit Account No. 50-4615.

Contents

REAL PARTY IN INTEREST

The real party in interest is Sirna Therapeutics Inc., a wholly owned subsidiary of Merck & Co., Inc.

RELATED APPEALS AND INTERFERENCES

Appeal No. 2009-2562, resulting from application No. 90/008,177 (Re-examination of US Patent 7,022,828). A copy of the Board's decision is attached as Appendix C.

Appeal No. TBD, resulting from application No. 10/720,448, currently pending before the Board.

STATUS OF CLAIMS

A Final Office Action was mailed on October 21, 2010. Claims 18-20 and 33-49 stand rejected and are presently pending. Claims 1-17 and 21-32 were previously canceled. The rejections of claims 18-20 and 33-49 are appealed with this submission. A copy of the claims on appeal is attached in Appendix A.

STATUS OF AMENDMENTS

No claims are amended.

SUMMARY OF THE CLAIMED SUBJECT MATTER

The invention provides certain chemically modified short interfering nucleic acid molecules having a sense strand and an antisense strand that mediate RNA interference. Each strand of the claimed nucleic acid molecules is between 18 and 24 nucleotides in length. The antisense strand is complementary to a target RNA sequence. The sense strand includes a terminal cap moiety at its 5' and 3' ends and the antisense strand includes a terminal cap moiety at its 3' end. Furthermore, 10 or more pyrimidine nucleotides of the sense strand and/or antisense strand are chemically modified with 2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro nucleotides. *See* claims 18 and 40; Specification at, *inter alia,* page 30, lines 2-7; and page 83, lines 5-7. See additionally Figures 18 and 19; Table I (beginning at page 227) and Table IV (page 239) for numerous examples of the presently claimed chemically modified nucleic acid molecules.

Each strand of the chemically modified short interfering nucleic acid molecules described above can be further modified with 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages. *See* claims 39 and 48; Specification at, *inter*

alia, page 30, lines 5-6; Figures 18 and 19; Table I (beginning at page 227) and Table IV (page 239).

The chemically modified short interfering nucleic acid molecules can have one or more ribonucleotides (claims 19 and 41) or alternately can comprise no ribonucleotides (claims 20 and 42). *See* specification at, *inter alia,* page 14, lines 14-16.

One or more of the pyrimidine nucleotides in the sense strand of the chemically modified short interfering nucleic acid molecule can be a 2'-O-methyl pyrimidine nucleotide. *See* claims 33 and 43; Specification at, *inter alia,* page 30, lines 2-4; Figures 18 and 19 (A, B, C); Table I (beginning at page 227) and Table IV (page 239) *e.g.,* "Stab 6" and "Stab 17".

One or more of the purine nucleotides in the sense strand of the chemically modified short interfering nucleic acid molecule can be a 2'-deoxy purine nucleotide. *See* claims 34 and 44; Specification at, *inter alia,* page 38, lines 7-10; Figures 18 and 19 (D, F); Table I (beginning at page 227) and Table IV (page 239) *e.g.,* "Stab 7".

One or more of the pyrimidine nucleotides in the sense and/or antisense strand of the chemically modified short interfering nucleic acid molecule can be a 2'-deoxy-2'-fluoro pyrimidine nucleotide. *See* claims 35, 36, 45 and 46; Specification at, *inter alia,* page 30, lines 2-4; Figures 18 and 19 (A, B, C, D, E, F); Table I (beginning at page 227) and Table IV (page 239) *e.g.,* "Stab 3", "Stab 4", "Stab 5", "Stab 7", "Stab 8", "Stab 11", "Stab 12", "Stab 13", "Stab 14", and "Stab 18".

One or more of the purine nucleotides in the antisense strand of the chemically modified short interfering nucleic acid molecule can be a 2'-O-methyl purine nucleotide. *See* claims 37 and 47; Specification at, *inter alia,* page 39, lines 18-21; Figures 18 and 19 (D, E); Table I (beginning at page 227) and Table IV (page 239) *e.g.,* "Stab 8", and "Stab 19".

The present invention also pertains to a composition comprising one of the molecules depicted above in a pharmaceutically acceptable carrier or diluent. *See* claims 38 and 49; Specification at, *inter alia,* page 19, lines 30-31.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issue on appeal is:

(I) Whether claims 18-20 and 33-49 are obvious under 35 U.S.C. § 103(a) over Elbashir et al. (EMBO J., 2001, 20(23):6877) in view of Matulic-Adamic et al. (US 5,998,203), Parrish et al. (Molecular Cell, 2000, 6:1077-87), and Crooke (US 5,898,031).

ARGUMENT

I. Claims 18-20 and 33-49 are inventive and not obvious

The present invention results from the discovery of chemically modified short interfering nucleic acid molecules that are both highly serum stable and active in mediating RNA interference by virtue of having 3' and 5' terminal cap modifications in the sense strand and 3' terminal cap modifications in the antisense strand combined with modification of pyrimidine nucleotides in both the sense and antisense strands with 2'-O-methyl, 2'-deoxy-2'-fluoro, or 2'-deoxy. The state of the art at the time of the invention provided stabilized siRNA molecules having limited 2'-deoxy modification of the 3'-terminal overhang regions only (*see* Elbashir, EMBO J., 2001, 20(23):6877). Attempts at more extensive modification, i.e., beyond the 3'-termini, was taught to "*reduce the ability of siRNAs to mediate RNAi, probably by interfering with protein association for siRNP assembly*" (*see* Elbashir, page 6885). The state of the art 3'-overhang modified siRNA molecules with retained RNAi activity as taught by Elbashir provide a useful research tool when used *in vitro*. However, when tested by Applicant, the 3'-overhang modified siRNA molecules of Elbashir et al. demonstrated a half life in human serum of 15 seconds, thus precluding their application *in vivo*. On the other hand, more extensively modified siRNA molecules of the instant invention, having terminal cap modifications at both 3' and 5'-ends of the sense strand and the 3'-end of the antisense strand combined with certain modified pyrimidine nucleotides in both sense and antisense strands, demonstrated serum half lives of up to 40 days (*see* Figure 3 of the instant application; *see* also Figure 3 of the earliest priority document USSN 60/358,580 filed February 20, 2002 which shows an increase in serum half life from 15 seconds to 72 hours). Surprisingly, the siRNA molecules of the instant invention also demonstrated similar or even improved activity compared to the state of the art molecules of Elbashir et al. when tested by Applicant (see discussion *infra* with respect to secondary indicia).

The present invention has significantly advanced the state of the art to allow for the use of serum stable synthetic chemically modified short interfering nucleic acid molecules with robust RNA interference activity for *in vivo* therapeutic applications. The Office has asserted that this significant advancement over the prior art resulted merely from "routine optimization", and alleges that the invention is *prima facie* obvious. Specifically, claims 18-20 and 33-49 stand rejected under 35 U.S.C. 103(a) as allegedly being obvious over Elbashir et al. (EMBO J., 2001, 20(23):6877) in view of Matulic-Adamic et al. (US 5,998,203), Parrish et al. (Molecular Cell, 2000, 6:1077-87) and

Crooke (US 5,898,031). *See* Office Action at page 3. Applicant respectfully traverses, and relies upon well established jurisprudence as discussed below to prove otherwise.

Applicant respectfully maintains that the presently claimed invention cannot be obvious for at least three reasons. First, one of skill in the art would *not have had any reasonable expectation of success* in practicing the claimed invention at the time of the invention because the prior art either taught away from the invention or indicated such a high level of unpredictability so as to preclude any reasonable expectation of success. Second, it is impermissible hindsight to conclude that the present invention is obvious because it would have been "obvious to try" using combinations of known modifications via "routine optimization", especially since the prior art gave *"no direction as to which of many possible choices is likely to be successful"* and offered *"only general guidance as to the particular form of the claimed invention or how to achieve it." In re O'Farrell,* 853 F.2d 894, 903 (Fed. Cir. 1988). Finally, even if a *prima facie* finding of obviousness could be established, the *failure of others*, along with the *surprising results* obtained in practicing the invention, would serve to effectively rebut any such presumption of obviousness.

1. No reasonable expectation of success

The Office alleges (*see* Final Office Action, page 9):

> *"Finally, one would have a reasonable expectation of success given that each of the modifications were known in the art at the time the invention was made to add benefits to antisense oligonucleotides, ribozymes, or siRNA duplexes, as evidenced by Elbashir et al., Matulic-Adamic et al., Parrish et al., and Crooke, wherein each of the molecules face the same challenges, and each of which can be improved with modifications.*

Applicant respectfully traverses and maintains that one of skill in the art at the time of the invention would not have any reasonable expectation of success in practicing the claimed invention because of the teaching away and high level of unpredictability provided by the cited art with respect to the ability of the claimed siRNA to mediate RNA interference due to mechanistic concerns. MPEP § 2143.03(VI) states that "[a] prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention." A reference will teach away when it suggests that the developments flowing from its disclosures are unlikely to produce the objective of the applicant's invention. *In re Gurley*, 27 F.3d 551, 553 (Fed. Cir. 1994). Here, because the Elbashir reference discloses problems with respect to more extensive modification beyond the 3'-terminal portions of a siRNA, and because these teachings are

based on the proposed RNAi mechanism as it was understood at the time of the invention, one of skill in the art would not be inclined to modify siRNA molecules as presently claimed nor have any reasonable expectation of success in doing so. This is due to a fundamental lack of predictability in using modifications that were known to benefit nucleic acid stability, yet demonstrated adverse effects when used beyond stabilizing the 3'-terminal single stranded regions of the siRNA duplex, presumably based on incompatibility with the RNAi mechanism. As such, the teachings of Elbashir et al. as a whole teach away from the instantly claimed invention that requires modification beyond the 3'-terminal overhang portions of a siRNA duplex.

Elbashir *et al.* described siRNA duplexes having from 9.5% to 100% of the nucleotides modified in each strand by replacing the 2'-hydroxyl group of said nucleotides with either 2'-deoxy or 2'-O-methyl. Figure 4 shows that when the two overhanging 3' nucleotides of each strand were modified with 2'-deoxy nucleotides, RNAi activity was maintained. The same result was found when the two additional nucleotides adjacent to the 3' overhangs of each strand were modified. However, when either one strand of the duplex was fully modified or both strands of the duplex were fully modified, RNAi activity was abolished. The authors summarize their findings in the Discussion section of the paper under the heading "The siRNA user guide" providing specific guidance to those skilled in the art for generating siRNA duplexes that are more palatable from a manufacturing cost perspective, and which may have enhanced resistance to nuclease degradation. The authors state on page 6885, with emphasis added, the following:

> *"Efficiently silencing siRNA duplexes are composed of 21 nt sense and 21 nt antisense siRNAs and* ***must*** *be selected to form a 19 bp double helix with 2 nt 3'-overhanging ends. 2'-deoxy substitutions of the 2 nt 3'-overhanging ribonucleotides do not affect RNAi, but help to reduce the costs of RNA synthesis and may enhance RNase resistance of siRNA duplexes.* ***More extensive 2'-deoxy or 2'-O-methyl modifications reduce the ability of siRNAs to mediate RNAi, probably by interfering with protein association for siRNP assembly.****"*

It should be noted that the teachings of the Elbashir reference have been reviewed by the BPAI who found that "[a] fair reading of [Elbashir]…is that more extensive 2'-deoxy or 2'-O-methyl modifications beyond the two nucleotide 3'-overhang reduces the ability of siRNAs to mediate RNAi." Appeal 2009-002562, at page 27. This fair reading is consistent with the position that modification as claimed in the instant application, and in particular, modification beyond the 3'-terminal regions of one or both strands of a siRNA molecule, is either expressly taught away from, or in the alternative, is highly

unpredictable in view of the teachings of Elbashir et al., especially since their conclusions were premised on mechanistic incompatibility, i.e., that more extensive modifications interfere with protein association for siRNP assembly.

Citing *DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.,* 567 F.3d 1314 (Fed. Cir. 2009), the Office recognized in the Examination Guidelines Update: Developments in the Obviousness Inquiry After KSR v. Teleflex (Notices) that,"[a]n inference that a claimed combination would not have been obvious is especially strong where the prior art's teachings undermine the very reason being proffered as to why a person of ordinary skill would have combined the known elements." Fed. Reg. 75:169 (September 1, 2010) page 53649. Clearly, one of skill in the art, having read the teachings of Elbashir et al., who warn against more extensive modification beyond the 3'-terminal regions due to proposed mechanistic concerns over the ability of the siRNA to associate with proteins required for RNAi, would certainly not have any reasonable expectation of success in arriving at active molecules with more extensive levels of modification. Specifically, in view of the teachings of Elbashir et al., one of skill in the art would not have any reasonable expectation of success with molecules having 10 or more modified pyrimidine nucleotides in the sense and/or antisense strand combined with 3' and 5' terminal cap modification of the sense strand and 3' terminal cap modification of the antisense strand because in aggregate, such modification would be expected to interfere with protein association for siRNP assembly. The reasonable expectation of success is diminished further when one considers additional modifications, such as the addition of phosphorothioate internucleotide linkages as per claims 39 and 48.

As discussed in the Examination Guidelines Update: Developments in the Obviousness Inquiry After KSR v. Teleflex (Notices), "[a] claimed combination of prior art elements may be nonobvious where the prior art teaches away from the claimed combination and the combination yields more than predictable results." Fed. Reg. 75:169 (September 1, 2010) page 53647 (citing *Crocs, Inc. v. U.S. Int'l Trade Comm'n.*, 598 F.3d 1294 (Fed. Cir. 2010)). The combination of features presently claimed, in view of the cited art that teaches against more extensive modification of siRNA beyond the 3'-overhangs due to mechanistic concerns, would not yield a predictable result in terms of maintaining RNAi activity and, accordingly, one of skill in the art at the time of the invention would not have any reasonable expectation of success. In fact, the instant invention demonstrates surprising and unexpected results (see discussion *infra* at page 17). Therefore, Applicant maintains that the Office's assertion that the instant invention is the result of routine optimization is clearly erroneous and ignores the teachings away

and unpredictability of the cited art with respect to the effect of more extensive modification on RNAi activity that was evident at the time of the instant invention. There is nothing in Matulic-Adamic et al., Parrish et al., or Crooke to remedy the shortcomings or teaching away that is evident in Elbashir et al.

The Office counters by alleging (*see* Advisory action, at page 2):

> *"Importantly, the instant claims are not directed to any specific target sequence and only require 10 pyrimidine modifications collectively between both strands. Therefore, depending on the target sequence, the diminimus of the instant claim breadth is only a difference between one modification on each strand because Elbashir et al teaches the incorporation of 8 modified positions. Therefore, although applicant continues to argue that Elbashir et al. teaches away, this simply is not the case"*

This argument is flawed however. Contrary to the Office's characterization of the present invention as differing from the closest prior art by one modification on each strand, the minimum requirement of claim 18 (with respect to modification) includes terminal cap modifications on both the 3'-end and the 5'-end of the sense strand and the 3'-end of the antisense strand, and 10 or more modified pyrimidine nucleotides (2'-OMe, 2'-F, or 2'-H) in the sense strand and antisense strand. The minimum requirement of claim 40 (with respect to modification) includes terminal cap modifications on both the 3'-end and the 5'-end of the sense strand and the 3'-end of the antisense strand, and 10 or more modified pyrimidine nucleotides (2'-OMe, 2'-F, or 2'-H) in the sense strand or antisense strand. The Office appears to be comparing apples to oranges in making its comparison. Elbashir simply teaches up to 4 consecutive 2'-deoxy modifications at the 3'-end of each strand. It follows that there are two elements missing in the Office's comparison to the closest prior art, i.e., (1) the 3' and 5' terminal cap modifications in the sense strand and 3' terminal cap modifications in the antisense strand, and (2) the pyrimidine modifications in the sense and/or antisense strand.

Therefore, even if one takes that position *arguendo* that, based on the teachings of Elbashir, one of skill in the art would envision designing a siRNA with at least 10 consecutively modified pyrimidine nucleotides (2'-OMe, 2'-F, or 2'-H) distributed between the 3'-ends of the sense and antisense strands of the duplex per claim 18 (e.g., 5 consecutively modified pyrimidines in the sense strand and 5 in the antisense strand, or various combinations such 1 and 9, 2 and 8, 3 and 7, 4 and 6, etc.), the Office ignores the requirement for 3' and 5' terminal cap modifications on the sense strand and 3' terminal cap modifications on the antisense strand. Even greater distortions are created when one envisions at least 10 consecutively modified pyrimidine nucleotides at the 3'-end of the

sense strand or antisense strand, in which essentially half of strand would be modified from the 3'-end onward. Furthermore, because having consecutive pyrimidine nucleotides at the 3'-end of one or both strands of the duplex (i.e., at least 5 in each strand or at least 10 in one strand) would place severe restrictions on the sequence used for any given siRNA, one of skill in the art would not be motivated to design such a duplex, let alone expect such a duplex to have RNA interference activity. ***Importantly, it is only in hindsight that such constructs can be envisioned, and even such a hindsight analysis ignores the terminal cap modifications to the 3' and 5' ends of the sense strand and the 3' end of the antisense strand***. (Emphasis added).

The Office further mischaracterizes the teachings of Elbashir in stating that "Elbashir et al. is evidence that modification is well tolerated in the terminal portions of the duplex, offering further motivation to modify the terminal regions." *See* Advisory Action, at page 2. However, there is nothing in Elbashir to teach or suggest that modifications are tolerated at both 3' and 5' terminal regions of either strand of the siRNA. On the contrary, the plain language of Elbashir indicates that such modifications would be expected to abrogate the ability of siRNAs to mediate RNAi, probably by interfering with protein association for siRNP assembly. There is simply no teaching or suggestion provided by Elbashir of any successful modification beyond the 2-4 nucleotides at the 3'-terminus of each strand. Any suggestion by the office that Elbashir provides motivation or any reasonable expectation of success in modifying both 3' and 5' terminal portions of the duplex is misplaced. A proper reading of Elbashir et al. reveals that RNAi activity of duplexes with more extensive modification beyond the 3'-terminal regions is taught away from, or is at least rendered highly unpredictable based on mechanistic concerns with respect to formation of the RISC complex (see Elbashir et al., page 6886).

> *We have been able to substitute eight ribose residues of a siRNA duplex by 2'-deoxyribose residues without substantial reduction of RNAi, although it should be noted the 2'-deoxy modifications were clustered at the 3' end of the siRNAs, including the 2 nt 3' overhangs. It is possible that the four 2'-deoxy modifications, which are located in the paired region at the end of the helix, do not affect the overall A-form helical structure and do not strongly compromise RISC formation. Complete modification of one or both siRNA strands by 2'-deoxyribose, however, abolished RNAi. Interestingly, substitution by 2'-O-methylribose, which adopts the ribose sugar pucker, also abolished RNAi, probably because methylation of the 2'-hydroxyls blocked hydrogen bond formation or introduced steric hindrance.*

The passage above, coupled with "The siRNA user guide," provides a strong teaching away and indicates a high level of unpredictability with respect to more extensive

modification of siRNA beyond the 3'-overhangs. "The siRNA user guide" states that more extensive modification with 2'-deoxy or 2'-O-methyl beyond the 3'-overhangs results in reduced RNAi activity "probably by interfering with protein association for siRNP assembly." The above discussion highlights this mechanistic concern and the *unpredictable effects of modification* beyond the 3' terminal overhang region. In fact, the Elbashir authors specifically address the issue of predictability in discussing the effect of modification on the ability of siRNA to form an A-form helical structure, the structure a native siRNA duplex adopts and which presumably is required for proper RISC formation. They found that 2'-deoxy modification clustered near the single stranded 3'-overhangs was tolerated, speculating that such modification did not affect the overall A-form helical structure of the duplex. Importantly, however, they further found that modification beyond the 3' overhangs with either 2'-deoxy or 2'-O-methyl nucleotides abolished RNAi activity. With regard to the 2'-O-methyl modifications, this was a particularly surprising result since 2'-O-methyl modifications were known to preserve A-form helical structure by adopting a ribose sugar pucker. Thus, with this finding, any predictability based on modification specific criteria previously thought to be well tolerated when incorporated into nucleic acid molecules is destroyed. One can only conclude that more extensive modification beyond the 3'-terminal overhang regions would have an adverse impact on RISC formation and RNAi activity and is, therefore, either expressly taught away from or, in the alternative, highly unpredictable based on the teachings of Elbashir et al. when read as a whole.

The issue of predictability with respect to modification of siRNA was addressed by the Board previously in Appeal 2009-002562. The BPAI recognized that the most effective siRNA molecules of the prior art are disclosed as having 3' overhangs. The 3' overhangs would reasonably be expected to be more susceptible to nuclease degradation by virtue of the being single stranded structures. In view of this, the Board stated that "[t]he combination of chemical modifications known to increase the nuclease resistance of nucleic acid molecules to siRNA molecules, particularly to the 2'-OH group of a single stranded 3' overhang, is likely to be obvious when it does no more than yield a predictable result." *See* Appeal 2009-002562, at pages 25-26. Here, the claimed siRNA molecules having terminal cap modifications at both 3' and 5'-ends of the sense strand and the 3'-end of the antisense strand, and having 10 or more modified pyrimidines in the sense and/or antisense strand, requires modification that clearly goes well beyond simply protecting the 2'-OH group of a single stranded 3'-overhang, and reaches well into the unpredictable territory that the Elbashir et al. authors caution away from based on

mechanistic concerns over steric interference with protein association for siRNP assembly. The Office reinforces this position in the December 30, 2010 Advisory Action by asserting that "capping as disclosed by Matulic-Adamic et al. would be reasonably expected to sterically interfere with the active site of a nuclease (see page 25 of decision, for example)". *See* Advisory Action, at page 2. While terminal capping can reasonably be expected to interfere with nuclease recognition, similar reasoning would also readily infer that 3' and 5'-terminus capping as disclosed by Matulic-Adamic et al. would be reasonably expected to sterically interfere with siRNP assembly as is required for proper RISC formation and RNA interference activity, per the teachings in the Elbashir reference when read as a whole.

In conclusion, Applicant agrees with the BPAI's characterization of the closest prior art in stating that "[a] fair reading of [Elbashir]…is that more extensive 2'-deoxy or 2'-O-methyl modifications beyond the two nucleotide 3'-overhang reduces the ability of siRNAs to mediate RNAi." Appeal 2009-002562, at page 27. There is nothing in the Matulic-Adamic, Parrish or Crooke references that remedies the unpredictability and teaching away that is evident in Elbashir et al. Because the instant invention requires terminal cap modifications on both the 3' and 5'-ends of the sense strand and the 3'-end of the antisense strand, *in addition to* 10 or more modified pyrimidines (2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro) in the sense strand and/or antisense strand, in view of the teachings of Elbashir et al., one of skill in the art at the time of the instant invention would simply not have any reasonable expectation of success due to the strong lack of predictability with respect to the ability of these molecules to mediate RNAi.

2. "Obvious to try" analysis fails to find obviousness

The Office continues to assert that "It was well within the technical grasp of the skilled artisan to combine chemical modifications that were known and routinely used to enhance stability of nucleic acid therapeutic molecules to arrive at molecules within [sic] he instantly claimed genus that would likely have activity, as it was known in the art to balance stability and activity via routinely testing different combinations/quantities of such modifications." *See* Advisory Action, at page 3. In doing so, Applicant maintains that the Office appears to rely on hindsight in putting forth the proposition that sooner or later, one of skill in the art would arrive at the instant invention by testing various combinations of modifications and locations "[i]t is the routine optimization of the placement of the modifications that is relied upon for determining activity of such molecules, as it was known to perform such routine testing, as evidenced by Elbashir et al." *See* Advisory Action, at page 2.

The Office is essentially arguing that the present invention would be "obvious to try" using known modifications and routine experimentation, and is therefore *prima facie* obvious. Applicant respectfully traverses. The Federal Circuit has clarified the standard for a finding of obviousness based on "obvious to try" in *In re Kubin*, 561 F.3d 1351, 1359 (Fed. Cir. 2009). While acknowledging that, as stated by the U.S. Supreme Court in *KSR International Co. v Teleflex Inc.,* a skilled artisan, when motivated by an unmet need, can look to combine elements within the scope of the prior art, it would be improper to hold a claim obvious when:

> *what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result; where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful*

or

> *what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.*

To hold a claim obvious under these situations would be, according to the Federal Circuit, "succumb[ing] to hindsight claims of obviousness" and erroneous. *Id.* Reaffirming its prior holdings in *In re O'Farrell,* 853 F.2d 894, 903 (Fed. Cir. 1988), the Federal Circuit explained that in order for an "obvious to try" inquiry to serve as the basis for obviousness, some direction in the prior art that would provide a reasonable expectation of success is still required. *See, O'Farrell,* at 903-04. Not only did the references cited herein provide no guidance as to what individual modifications when used "more extensively" can result in siRNA molecules that are both active and stable, they in fact indicate that more extensive incorporation of these modifications into siRNA is detrimental, or at least highly unpredictable based on the proposed mechanism of action, i.e. ***by interfering with protein association for siRNP assembly***. (Emphasis added) The prior art references therefore provide no guidance or any level of predictability that would allow one of skill in the art to have any reasonable expectation of success using *the combination of features* as presently claimed. Therefore, even an "obvious to try" inquiry fails to result in a finding of obviousness as one of skill in the art would simply have ***no reasonable expectation of success*** in practicing the instantly claimed invention as discussed *supra*.

A reading of the cited prior art reveals a vast number of possible modifications that were available to one of skill in the art at the time of the instant invention. The Office, in hindsight, attempts to oversimplify the criteria as being limited to only two choices, i.e. modification of purine vs. pyrimidine nucleotides in stating that "[i]t is certainly within the realm of routine optimization/design choice to incorporate the modifications at a purine or a pyrimidine, given that there are only two choices." *See* Advisory Action, at page 3. However, the claims require specific selections from at least *4 different criteria*: (1) the extent/number of modifications, i.e. terminal cap modifications at both the 3' and 5'-ends of the sense strand and the 3'-end of the antisense strand in addition to 10 or more pyrimidine modifications in the sense and/or antisense strand; (2) the types of modifications, i.e. 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro; (3) the positions of certain modifications, i.e. CAPs both 3' and 5'-ends of the sense strand and 3'-end of the antisense strand; and (4) the type of nucleotide that is modified irrespective of its position within the duplex, i.e. specific modification of pyrimidine nucleotides. Therefore, the present invention could not have possibly arisen from routine optimization.

Even if one takes the position that routine testing with known modifications and known assays would ***eventually*** lead one of skill in the art to the presently claimed invention, this would be insufficient to establish a *prima facie* case of obviousness for at least two reasons. First, the references cited by the Office fail to give any indication of which parameters were critical to success, and in many instances taught away from the claimed modifications when applied more extensively. Second, at the time of the present invention, RNAi was a new technology and the experiences of the antisense/ribozyme arts at most gave general guidance as to the types of modifications one could apply to a short dsRNA molecule, providing merely a large selection of possibilities to choose from. These known modifications were individually demonstrated by those who first studied short dsRNA in the field to be sometimes feasible with limited application, but more often than not were incompatible with RNAi activity due mechanistic concerns, i.e., incompatibility with the siRNP protein machinery that is required to mediate RNAi. That unpredictability grows only larger if the known modifications were applied more extensively, and in combination, as is presently claimed. Thus, although numerous types of modifications were known in the art, this was not a case of testing a finite number of identified, predictable solutions. ***"In such circumstances, where a defendant merely throws metaphorical darts at a board filled with combinatorial prior art possibilities, courts should not succumb to hindsight claims of obviousness."*** *Kubin,* at 1359.

Therefore, this is not an instance where the prior art contained detailed enabling methodology for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, ***and evidence suggesting that it would be successful.*** Rather, it is an instance where the prior art provides no direction as to which of many possible choices is likely to be successful and only general guidance as to the particular form of the claimed invention or how to achieve it. Most importantly (as addressed previously), the prior art, by teaching away from (or at least rendering highly unpredictable) more extensive modification, evidenced such a high level of unpredictability to preclude any reasonable expectation of success in practicing the claimed invention. Applicant's arguments do not rest on an absolute predictability of success, but rather point to a fundamental lacking of even a reasonable expectation of success in view of mechanistic concerns over modifications extending beyond 2'-deoxy modification of the 3'-overhangs. Any finding of obviousness under the "obvious to try" standard is therefore improper under the jurisprudence of *Kubin* and *O'Farrell.*

3. Secondary indicia preclude any finding of obviousness

Applicant maintains that no *prima facie* finding of obviousness can stand in view of the lack of motivation or any reasonable expectation of success that is evident from a plain reading of the cited art, and that even an "obvious to try" analysis fails because of the lack of guidance and/or predictability offered by the prior art. However, even if a *prima facie* showing of obviousness could be established, such a finding is effectively rebutted due to secondary considerations. It is well established that "evidence rising out of the so-called 'secondary considerations' must always when present be considered en route to a determination of obviousness". *Stratoflex, Inc. v. Aeroquip Corp*, 713 F.2d 1530, 1538 (Fed. Cir. 1983). Secondary considerations include the failure of others and unexpected results. MPEP 716.01(a). Specifically, (1) the failure of others, coupled with (2) the surprising results obtained using the instant invention, are a clear and irrefutable demonstration of non-obviousness with respect to the presently claimed invention.

The instant invention provides double stranded nucleic acid molecules that are both highly serum stable and potent in mediating RNA interference, both *in vitro* and *in vivo*. The closest prior art is the Elbashir reference cited herein. The authors of Elbashir, armed with all of the knowledge proffered by the prior art with respect to chemical modification of nucleic acids (including the work published in the Parrish reference, along with the prior teachings of Matulic-Adamic) conducted extensive characterization and analysis of double stranded nucleic acid molecules with respect to optimized activity. Those authors published *'The siRNA user guide'* with respect to their findings. They

attempted to stabilize double stranded nucleic acid molecules, but *failed* in providing molecules that are both serum stable and active (see discussion *supra* and in more detail below with respect to **Figure 3** of the instant application and priority applications). In fact, Elbashir taught that double stranded nucleic acid molecules that were "more extensively" modified beyond 2'-deoxy modification of the 3'-terminal nucleotide positions "reduce the ability of siRNAs to mediate RNAi, probably by interfering with protein association for siRNP assembly." Elbashir, at page 6885, under *'The siRNA user guide.'* As such, Elbashir failed to arrive at a chemically modified double stranded nucleic acid molecule that is both serum stable and has retained (let alone improved or potent) activity and concluded that more extensive modifications were not favorable because of mechanistic concerns over the inability of such more extensively modified siRNA to interact with the RNAi protein machinery.

The instant invention is a departure from the teachings of Elbashir's *'The siRNA user guide'* and provides double stranded nucleic acid molecules having features that impart a high level of serum stability yet maintain significant, or even improved, RNAi activity compared to those of the prior art (see **Figures 3, 10, 11, 12, 13, 14, 15, 26, 29, 30, 39, 40, 41, 77, 80, 81, 82, 83, 84, 85, 86, and 87** and **Table I and IV** of the instant application, specific examples of which are described in greater detail below). These features are presently claimed, i.e. terminal cap modifications on both the 3'-end and the 5'-end of the sense strand and the 3'-end of the antisense strand, and 10 or more modified pyrimidine nucleotides (2'-OMe, 2'-F, or 2'-H) in the sense strand and (claim 18) /or (claim 40) antisense strand. Application of these claimed features results in double stranded nucleic acid molecules with surprising and unexpected properties as described below.

The Office asserts that "in the instant case applicant is not claiming any specific combination or modification schematic that produces an unexpected result, but is rather claiming a huge genus of possible molecules wherein molecules within the genus are certainly considered obvious in terms of the prior art" *See* Advisory Action, at page 2. Applicant respectfully disagrees with this highly conclusory characterization of the invention and maintains that the invention, when properly understood, is directed to a specific modification schematic that can be applied to any double stranded nucleic acid sequence as described in the specification, and which provides unexpected results. For example, application of the features of independent claim 18 or 40 to any duplex sequence will result in a specific structure with well defined features that include: (1) the length of each strand being between 18 and 24 nucleotides; (2) terminal cap

modifications at both 3' and 5'-ends of the sense strand and the 3'-end of the antisense strand; and (3) the modification of 10 or more pyrimidine nucleotides of the sense strand and/or antisense strand with 2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro nucleotides.

Application of the claimed features to a double stranded nucleic acid sequence of interest as taught by the instant application provides surprising and unexpected results. These unexpected results are clearly taught by the application as filed. Examiners must consider comparative data in the specification which is intended to illustrate the claimed invention in reaching a conclusion with regard to the obviousness of the claims. *In re Margolis*, 785 F.2d 1029, 228 USPQ 940 (Fed. Cir. 1986). For example, inspection of **Figure 3** of the instant application shows a direct comparison of the state of the art at the time of the invention (modified Elbashir duplex, Figure 4 on page 6882 of Elbashir *et al.*) to duplexes of the instant invention in terms of nuclease stability. The Elbashir duplex, having 3'-terminal 2'-deoxy modifications (SEQ ID NOs: 394 and 395), when tested in human serum, has a half life (T ½) of *15 seconds*. The duplexes of the instant invention however, having 3' and 5'-caps combined with the enumerated pyrimidine modifications, show dramatically improved nuclease stability: T ½ of *138 minutes* for SEQ ID NOs: 396 and 397; T ½ of *3.7 days* for SEQ ID NOs: 396 and 398; T ½ of *72 minutes* for SEQ ID NOs: 396 and 399; T ½ of *40 days* for SEQ ID NOs: 396 and 400; and T ½ of *32 days* for SEQ ID NOs: 396 and 401.

Additionally, the RNAi activity of duplexes of the invention having 3' and 5' caps combined with 2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro pyrimidine modifications in the sense strand and/or antisense strand, is surprisingly *comparable to or even improved* when compared to a control duplex of the prior art. See for example **Figure 14**, in which the siGL2 control (Elbashir duplex) is compared to duplexes of the invention having a "Stab 6" (see Table IV) sense strand (sequence 30222, SEQ ID NO: 373) consisting of 3' and 5'-terminal caps with 2'-O-methyl and 2'-deoxy pyrimidine modifications and various "Stab 5" (Table IV) antisense strands, all having 2'-deoxy-2'-fluoro and 2'-deoxy pyrimidine modifications and 3'-terminal caps (sequence 30546, SEQ ID NO: 386; sequence 30224, SEQ ID NO: 374; sequence 30551, SEQ ID NO: 387; sequence 30557, SEQ ID NO: 388, and sequence 30558, SEQ ID NO: 389). Also, see for example **Figure 15**, in which the siGL2 control (Elbashir duplex) is compared to duplexes of the invention having a "Stab 4", "Stab 8" or "Stab 7" (Table IV) sense strand (sequence 30063, SEQ ID NO: 372; sequence 30434, SEQ ID NO: 384; and sequence 30435, SEQ ID NO: 385 respectively) all consisting of 3' and 5'-terminal caps with 2'-deoxy, 2'-deoxy-2'-fluoro or 2'-O-methyl pyrimidine modifications and a "Stab 8" (Table IV)

antisense strand having 2'-deoxy-2'-fluoro pyrimidine and 3'-terminal cap modifications (sequence 30430, SEQ ID NO: 375). As shown in these figures, the activity of the serum stable double stranded nucleic acid molecules of the invention is an *unexpected finding* in view of the teachings of the closest prior art.

The unexpected results, contrary to the teaching of the prior art, are also clearly exemplified in **Figures 28, 29, and 30**, in which the RNAi activity of various duplexes of the invention (Stab 4/5; Stab 7/8, and Stab 7/11 respectively), all having sense strands with 3' and 5'-terminal caps combined with 2'-deoxy and 2'-deoxy-2'-fluoro pyrimidine modifications with ribonucleotide (Stab 4, Table IV) or 2'-deoxy (Stab 7, Table IV) purines and antisense strands having 2'-deoxy and 2'-deoxy-2'-fluoro pyrimidine modifications with 3'-terminal cap modifications and with ribonucleotide (Stab 5, Table IV), 2'-O-methyl (Stab 8, Table IV) or 2'-deoxy (Stab 11, Table IV) purines) are compared to an all RNA duplex control in inhibiting HBV gene expression in a dose response time course study (note, all sequences for the constructs in **Figures 28, 29, and 30** are described in **Table I**). As shown in **Figures 28, 29, and 30**, the extensively modified duplexes of the invention all show comparable activity to the all RNA control at day 3, and *improved* activity at day 6 and day 9 time points.

As is clearly shown in **Figures 3, 14, 15, 28, 29, and 30** (amongst others), ***the double stranded nucleic acid molecules of the invention are significantly more stable than the double stranded nucleic acid molecules of the prior art, and surprisingly have retained or improved activity over the prior art molecules that allow these molecules to function as therapeutic modalities***. (Emphasis added) The chemically modified duplexes of the instant invention are a significant and inventive advancement over the teachings of the closest prior art Elbashir reference, which teaches that "more extensive" modification is detrimental to RNAi activity on mechanistic grounds because of impaired association with the RNAi protein machinery, and where attempts to more extensively modify such molecules resulted in *abolished* activity. Thus, even if the Office were able to make a *prima facie* showing of obviousness (which is not the case), the failure of others combined with the surprising and unexpected results as taught by the application as filed and the priority documents, unequivocally preclude any finding of obviousness.

II. Conclusions

The instant claims are patentable. Applicant therefore respectfully requests withdrawal of the standing rejections and allowance of the claims.

Respectfully submitted,

Date: March 14, 2011 By: **/Peter Haeberli/**
Peter Haeberli
Reg. No. 52,980
Attorney for Applicant

APPENDIX A

CLAIMS ON APPEAL

1. - 17. (Canceled)

18. (Previously presented) A chemically modified double stranded short interfering nucleic acid molecule that mediates RNA interference, wherein:

a) the double stranded nucleic acid comprises a sense strand and an antisense strand;

b) each strand is independently 18 to 24 nucleotides in length;

c) the sense strand includes a terminal cap moiety at its 5'- and 3'-ends and the antisense strand includes a terminal cap moiety at its 3'-end; and

d) 10 or more pyrimidine nucleotides of the sense strand and antisense strand are chemically modified with 2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro nucleotides.

19. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein said double stranded nucleic acid molecule comprises no ribonucleotides.

20. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein said double stranded nucleic acid molecule comprises one or more ribonucleotides.

21. - 32. (Canceled)

33. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein one or more pyrimidine nucleotides present in the sense strand are 2'-O-methyl pyrimidine nucleotides.

34. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein one or more purine nucleotides present in the sense strand are 2'-deoxy purine nucleotides.

35. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein one or more pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

36. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein one or more pyrimidine nucleotides present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

37. (Previously presented) The double stranded nucleic acid molecule of claim 18, wherein one or more purine nucleotides present in said antisense strand are 2'-O-methyl purine nucleotides.

38. (Previously presented) A composition comprising the double stranded nucleic acid molecule of claim 18 and a pharmaceutically acceptable carrier or diluent.

39. (Previously presented) The double stranded nucleic acid molecule of claim 18, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages.

40. (Previously presented) A chemically modified double stranded short interfering nucleic acid molecule that mediates RNA interference, wherein:

a) the double stranded nucleic acid comprises a sense strand and an antisense strand;

b) each strand is independently 18 to 24 nucleotides in length;

c) the sense strand includes a terminal cap moiety at its 5'- and 3'-ends and the antisense strand includes a terminal cap moiety at its 3'-end; and

d) 10 or more pyrimidine nucleotides of the sense strand or antisense strand are chemically modified with 2'-deoxy, 2'-O-methyl, or 2'-deoxy-2'-fluoro nucleotides.

41. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein said double stranded nucleic acid molecule comprises no ribonucleotides.

42. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein said double stranded nucleic acid molecule comprises one or more ribonucleotides.

43. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein one or more pyrimidine nucleotides present in the sense strand are 2'-O-methyl pyrimidine nucleotides.

44. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein one or more purine nucleotides present in the sense strand are 2'-deoxy purine nucleotides.

45. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein one or more pyrimidine nucleotides present in the sense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

46. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein one or more pyrimidine nucleotides present in said antisense strand are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

47. (Previously presented) The double stranded nucleic acid molecule of claim 40, wherein one or more purine nucleotides present in said antisense strand are 2'-O-methyl purine nucleotides.

48. (Previously presented) The double stranded nucleic acid molecule of claim 40, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages.

49. (Previously presented) A composition comprising the double stranded nucleic acid molecule of claim 40 and a pharmaceutically acceptable carrier or diluent.

APPENDIX B

EVIDENCE APPENDIX

None

APPENDIX C

RELATED PROCEEDINGS APPENDIX

See attached decision for Appeal No. 2009-2562, resulting from application No. 90/008,177 (Re-examination of US Patent 7,022,828).

APPENDIX D

AMENDMENTS IN THE CLAIMS

None

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte SIMA THERAPEUTICS, INC.,
Patent Owner and Appellant

Appeal 2009-002562
Reexamination Control 90/008,177
Patent 7,022,828
Technology Center 3900

Decided:[1] May 26, 2009

Before CAROL A. SPIEGEL, ROMULO H. DELMENDO, and JEFFREY FREDMAN, *Administrative Patent Judges*.

SPIEGEL, *Administrative Patent Judge*.

DECISION ON APPEAL

I. Statement of the Case

Appellant appeals under 35 U.S.C. §§ 134(b) and 306 from an Examiner's final rejection of all pending claims, claims 1-9 and 14-16.[2] We have jurisdiction under 35 U.S.C. §§ 134(b) and 306. We AFFIRM.

---

[1] The two-month for filing an appeal or commencing a civil action, as recited in 37 C.F.R. § 1.304, begins to run from the decided date shown on this page of the decision. The time period does not run from the Mail Date (paper delivery) or Notification Date (electronic delivery).

The subject matter on appeal is directed to a double stranded ("ds") siRNA molecule that down regulates expression of an IKK-γ gene and which has been modified with at least one 2'-O-methyl group and at least one 2'-fluoro group to protect the siRNA from nuclease degradation. Each strand of the siRNA is about 18 to about 28 nucleotides ("nt") in length. Claims 1, 3, 4, and 7 are illustrative and read (App. Br.[3] 25-26):

> 1. A chemically modified double stranded siRNA molecule that down regulates expression of an IKK-gamma gene via RNA interference (RNAi), wherein: a) each strand of said siRNA molecule is independently about 18 to about 28 molecules in length; b) one strand of said siRNA molecule comprises nucleotide sequence having sufficient complementarity to an RNA of said IKK-gamma gene for the siRNA to direct cleavage of said RNA via RNA interference; and (c) wherein said siRNA molecule comprises 2'-O-methyl and 2'-fluoro modifications.
>
> 3. The siRNA molecule of claim 1, wherein said siRNA molecule is assembled from two separate oligonucleotide fragments wherein a first fragment comprise the sense strand and a second fragment comprises the antisense strand of said siRNA molecule.
>
> 4. The siRNA molecule of claim 3, wherein said sense strand is connected to the antisense strand via a linker molecule.

---

[2] Appellant cancelled claim 13 in its Reply Brief under 37 C.F.R. § 41.41 filed 5 September 2008 ("Reply Br."), as acknowledged by the Examiner in a miscellaneous communication mailed 17 September 2008.
[3] Supplemental Brief on Appeal filed 13 June 2008 ("App. Br.").

> 7. The siRNA molecule of claim 3, wherein said second fragment comprises a terminal cap moiety at a 5'-end, a 3'-end, or both of the 5' and 3' ends of said second strand.

The Examiner has rejected

(A) claims 1-3, 14, and 16 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I[4] and Tuschl II[5] in view of Yamaoka,[6] Smahi,[7] and Krappmann[8] (Ans.[9] 5-8);

(B) claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I, Tuschl II, Yamaoka, Smahi, and Krappmann taken further in view of Agrawal[10] (Ans. 8);

---

[4] Published Patent Application US 2002/0086356 A1, *RNA Sequence-Specific Mediators of RNA Interference*, published 4 July 2002, based on application 09/821,832, filed 30 March 2001, by Tuschl et al. ("Tuschl I").
[5] International Publication WO 02/44321 A2, *RNA Interference Mediating RNA Small RNA Molecules*, published 6 June 2002, based on international application PCT/EP01/13968, filed 29 November 2001, by Tuschl et al., which claims priority benefit of US provisional application 60/279,661, filed 30 March 2001 ("Tuschl II").
[6] Yamaoka et al., "Complementation Cloning of NEMO, a Component of the IκB Kinase Complex Essential for NK-κB Activation," *Cell*, 93 (26 June 1998): 1231-1240 ("Yamaoka").
[7] Smahi et al., "Genomic Rearrangement in *NEMO* impairs NF-κB activation and is the cause of incontinentia pigmenti," *Nature*, 405 (25 May 2000):466-472 ("Smahi").
[8] Krappmann et al., "The IκB Kinase (IKK) Complex is Tripartite and Contains IKKγ but not IKAP as a Regular Component," *The Journal of Biological Chemistry*, 275 (22 September 2000):29779-29787 ("Krappmann").
[9] Examiner's Answer mailed 7 July 2008 ("Ans.").
[10] International Publication WO 94/01550, *Self-Stabilized Oligonucleotides as Therapeutic Agents*, published 20 January 1994, based on international

(C) claims 7 and 8 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I, Tuschl II, Yamaoka, Smahi, and Krappmann taken further in view of Matulic-Adamic[11] (Ans. 9);

(D) claims 1-3 under 35 U.S.C. § 103(a) as unpatentable over Kenwrick[12] in view of Tuschl I and Tuschl II (Ans. 9-11);

(E) claims 1-3 and 14-16 under 35 U.S.C. § 103(a) as unpatentable over Elbashir,[13] Tuschl II, and Bass[14] in view of Yamaoka, Krappmann, and Smahi (Ans. 11-14);

(F) claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, and Smahi further in view of Agrawal (Ans. 14-15);

(G) claims 7-8 under 35 U.S.C. § 103(a) as unpatentable over Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, and Smahi further in view of Matulic-Adamic (Ans. 15);

---

application PCT/US93/06326, filed 2 July 1993, by Agrawal et al. ("Agrawal").

[11] US Patent 5,988,203, *Enzymatic Nucleic Acids Containing 5'- and/or 3'Cap Structures*, issued 7 December 1999, to Matulic-Adamic et al. ("Matulic-Adamic").

[12] US Patent 6,824,972 B2, *Diagnosis and Treatment of Medical Conditions Associated with Defective NFKappa B (NF-κB) Activation*," issued 30 November 2004, based on application 09/863,049, filed 22 May 2001, to the Kenwrick et al. ("Kenwrick").

[13] Elbashir et al., "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells," *Nature*, 411 (24 May 2001):494-498 ("Elbashir").

[14] B. Bass, "The short answer," *Nature*, 411 (24 May 2001):428-429 ("Bass").

(H) claims 1-3, 9, and 14-16 under 35 U.S.C. § 103(a) as unpatentable over Fire[15] and Tuschl II in view of Yamaoka, Krappmann, and Smahi (Ans. 16-18);

(I) claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Fire, Tuschl II, Yamaoka, Krappmann, and Smahi further in view of Agrawal (Ans. 18-19); and,

(J) claims 7-8 under 35 U.S.C. § 103(a) as unpatentable over Fire, Tuschl II, Yamaoka, Krappmann, and Smahi further in view of Matulic-Adamic (Ans. 19-20).[16]

The rejections fall into two groups. In the first group, the Examiner relies on (a) Tuschl I, Elbashir, or Fire as teaching sequence-specific inhibition of gene expression by dsRNA (RNA interference or "RNAi"), specifically by siRNA, (b) Tuschl II as teaching 19-21 nucleotide siRNA wherein the 2' OH group of a ribose is replaced by an OR, wherein R is C_{1-6} alkyl, or a fluoro to improve stability, (c) Yamaoka as teaching the cDNA sequence of an IKK-γ gene, and (d) Krappmann and Smahi each as teaching the relationship between the IKK-κ gene and NF-κB activation. The Examiner further relies on Agrawal and Matulic-Adamic as teaching additional methods of improving the stability of RNA strands from degradation by nucleases. In the second group, the Examiner relies on Kenwrick as teaching methods of treating NF-κB related medical conditions

---

[15] US Patent 6,506,559 B1, *Genetic Inhibition by Double-Stranded RNA*, issued 14 July 2003, based on application 09/215,257, to Fire et al. ("Fire").
[16] The Examiner withdrew the rejection of claims 1-9 and 13-16 under 35 U.S.C. §§ 112, first paragraph (written description), and 305 (enlarging the scope of the claimed invention) (Ans. 2-3). Cancellation of claim 13 renders its rejections under 35 U.S.C. § 103(a) moot.

with sequences 100% complementary to an IKK-γ gene and Tuschl I and II as teaching the use of modified siRNA to block mRNA translation *in vivo*.

Appellant argues that the Examiner has failed to give any reason for providing 2'-O-methyl *and* 2'-fluoro modifications on the same siRNA, especially since Tuschl II is alleged to teach avoiding 2'-O-methyl modifications of the siRNA. Appellant further argues that Yamaoka fails to disclose the cDNA sequence encoding an IKK-γ gene and that the Examiner has failed to provide any reason for down regulating IKK-γ gene expression. Appellant finally argues that the teachings of Agrawal and Matulic-Adamic are directed to antisense and ribozyme interference RNA technology, respectively, and therefore are irrelevant to siRNA interference technology.

At issue is whether Appellant has shown the Examiner reversibly erred in (i) finding that Yamaoka disclosed the cDNA sequence of an IKK-γ gene, (ii) concluding that an ordinarily skilled artisan would have had both a motivation to and a reasonable expectation of success of providing an siRNA molecule with at least one 2'-O-methyl group and at least one 2'-fluoro group, (iii) failing to provide a reason for down regulating an IKK-γ gene, (iv) concluding that it would have been obvious to join a sense and an anti-sense RNA strand into a single ds siRNA via a linker molecule based on the teachings of Agrawal, (v) concluding that it would have been obvious to cap either or both ends of the antisense strand based on the teachings of Matulic-Adamic, and (vi) concluding that Kenwrick provided a teaching or suggestion of down regulating IKK-γ gene expression.

Since Appellant has provided separate patentability arguments for claim groupings 4-6 and 7-8, we decide this appeal on the basis of claims 1,

4 and 8. 37 C.F.R. § 41.37(c)(1)(vii). We also note that the Evidence Appendix of the principal brief does not cite to or provide any evidence being relied upon by Appellant in this appeal. 37 C.F.R. § 41.37(c)(2)(ix).

II. Findings of Fact ("FF")

The following findings of fact are supported by a preponderance of the evidence of record.

A. RNA interference

[1] The central dogma of molecular biology comprises three processes: replication (the copying of parental DNA to form daughter DNA molecules with identical nucleotide sequences), transcription (the process by which parts of the genetic message encoded in DNA are precisely copied into RNA), and translation (whereby the genetic message encoded in messenger RNA ("mRNA") is translated, i.e., expressed, into a protein with a particular amino acid sequence) (see e.g., Lehninger[17] 921-922).

[2] As stated by Bass, "[o]ne way of seeing what a gene does is to block its messenger RNA and note the effects" (Bass abstract).

[3] Several strategies can be used to achieve gene-specific inhibition, including single strand nucleic acid mediated antisense[18] technology and dsRNA mediated RNA interference (Fire 1: 63-67; 4:20-28).

[4] RNA interference ("RNAi") is a cellular process by which a dsRNA sequence-specifically blocks the expression of a gene (Fire 1:17-19;

---

[17] Lehninger PRINCIPLES OF BIOCHEMISTRY, fourth edition, W.H. Freeeman and Company, New York (2005) ("Lehninger") (copy enclosed).
[18] Antisense technology involves a single stranded antisense oligonucleotide pairing with its complementary mRNA to block translation (Fire 1:63-2:20).

Tuschl I ¶3; Bass 428, col. 1, ¶2; Elabshir 494 abstract; Tuschl II 1:11-26).

[5] According to Fire "dsRNA is at least 100-fold more effective than ... antisense RNA in reducing gene expression" (Fire 3:30-32).

[6] Similarly, according to Tuschl I and Elbashir, "siRNAs are effective at concentrations that are several orders of magnitude below the concentrations applied in conventional antisense or ribozyme gene targeting experiments" (Tuschcl I ¶148; Elbashir p. 496, col. 1, ¶2).

[7] According to Fire, the dsRNA (i) may be formed by a single self-complementary RNA strand or two complementary RNA strands (i.e., sense and antisense strands), (ii) preferably contains a nucleotide sequence greater than 90% identical to a portion of the gene being targeted for inhibition, (iii) may include modifications to the phosphate-sugar backbone or the nucleoside, and (iv) is at least 25 base pairs long (Fire 4:41-46; 7:31-32, 42-44, 53-54, 64-67; 26:55-59 (claim 10)).

[8] According to Fire, the dsRNA may be used to inhibit expression of a gene associated with disease causation or symptoms or to identify gene function in an organism (Fire 9:65-10:7; 10:26-28; 12:17-20).

[9] According to Tuschl I,

> RNAi is envisioned to begin with cleavage of the dsRNA to 21-23 nt products by a dsRNA-specific nuclease ... These short dsRNAs might then be dissociated with an ATP-dependent helicase ... to 21-23 nt asRNAs [i.e., antisense RNAs] that could then target the mRNA for cleavage. The short asRNAs are imagined to remain associated with the RNAi-specific proteins ... originally bound by

> the full-length dsRNA ... Finally, a nuclease ... would cleave the mRNA. [Tuschl I ¶36.]

[10] Thus, Tuschl I is directed 21-23 nt dsRNA molecules useful as sequence-specific mediators of RNAi to inhibit mRNAs that encode proteins associated with or causative of a disease or an undesirable condition (Tuschl I ¶57).

[11] In other words, according to Tuschl I, if the sequence of the gene to be targeted is known, any dsRNA can be used to mediate RNAi provided that it has sufficient homology to the targeted gene (Tuschl I ¶¶ 57, 59).

[12] Tuschl I also showed that 21 nt siRNA duplexes were able to specifically inhibit a target gene expression without activating an interferon response (Tuschl I ¶144).

[13] A preferred embodiment of Tuschl I comprises 21 nt strands with 2 nt overhangs at both 3' ends of the RNA, wherein the 3' overhangs can be modified, e.g., by 2' (deoxy) thymidine substitution, to enhance the stability of the siRNA (Tuschl I ¶¶55, 148).

[14] According to Tuschl I, the absence of a 2' OH group enhances the nuclease resistance of the 3' overhang *in vitro* (Tuschl I ¶55).

[15] Elbashir showed that 21 and 22 nt siRNA duplexes mediated sequence-specific mRNA degradation, without activating the interferon system as do dsRNAs having more than 30 base paired nucleotides (Elbashir abstract; p. 495, col. 1, ¶2; p. 496, col. 1, ¶3).

[16] Specifically, Elbashir synthesized 21-nt siRNA duplexes comprising sense and antisense strands with 2-nt 3' overhangs comprising 2' (deoxy) thymidine because the thymidine overhang reduces the cost

of RNA synthesis and may enhance the nuclease resistance of the siRNA (¶ bridging pp. 495-496; Figure 1).[19]

[17] According to Bass, although RNAi is routine in laboratories to study a wide range of organisms, Tuschl (explicitly referring to Elbashir) describes research that paves the way for successful RNAi use in mammalian cells, namely that siRNAs shorter than about 30 bps mediate a sequence-specific response whereas longer dsRNAs mediate a global, nonspecific response (Bass, p. 428; Figure 1).

B. RNA strand modifications

[18] Tuschl II discloses 19-25 nt siRNA duplexes capable of mediating RNAi and/or DNA methylation, wherein the most effective dsRNAs comprise two 21 nt strands which base paired such that 1-3, especially 2 nt 3' overhangs are present on both ends of the dsRNA (Tuschl II 3:18-24; 4:18-21).

[19] According to Tuschl II,

> [t]he target RNA cleavage reaction guided by siRNAs is highly sequence-specific. However, not all positions of a siRNA contribute equally to target recognition. Mismatches in the center of the siRNA duplex are most critical and essentially abolish target RNA cleavage. In contrast, the 3' nucleotide of the siRNA strand (e.g., position 21) that is complementary to the single-stranded target

[19] DNA and RNA differ in three main ways. First, RNA is usually a single-stranded molecule in most of its biological roles, whereas DNA is usually double-stranded. Second, RNA contains a ribose sugar, whereas DNA contains a deoxyribose sugar (i.e., deoxyribose does not have an OH group attached to the 2' position of the sugar ring). Third, the complementary base to adenine in RNA is uracil, not thymine as it is in DNA.

> RNA, does not contribute to specificity of the target recognition. Further, the sequence of the unpaired 2-nt 3' overhang of the siRNA strand with the same polarity as the target RNA is not critical for target RNA cleavage as only the antisense siRNA strand guides target recognition. Thus, from the single-stranded overhanging nucleotides only the penultimate position of the antisense siRNA (e.g., position 20) needs to match the targeted sense mRNA. [Tuschl II 4:23-5:2.]

[20] In other words, according to Tuschl II, the cleavage sites for both sense and antisense target RNAs are located in the middle of the region spanned by the siRNA duplexes (Tuschl II 47:4-5).

[21] Tuschl II discloses stabilizing the 3' overhangs against degradation to enhance *in vivo* and *in vitro* stability of the siRNAs (Tuschl II 5:4-9).

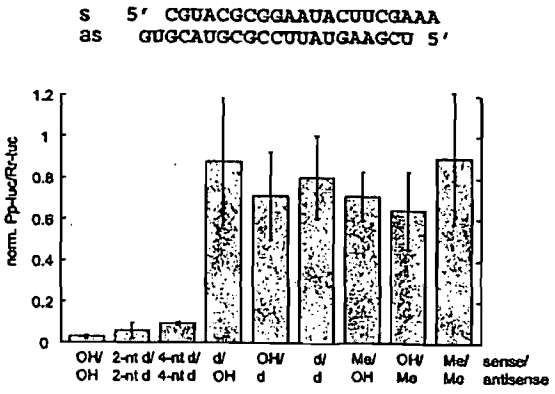
[22] According to Tuschl II, absence of a 2' OH group enhances the nuclease resistance of the overhang *in vitro* (Tuschl II 5:11-13).

[23] For example, Tuschl II discloses that "[i]n preferred sugar-modified ribonucleotides the 2' OH-group is replaced by a group selected from H, OR, R, halo, SH, SR, NH_2, NHR, NR_2 or CN, wherein R is C_1-C_6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I" (Tuschl II 5:31-6:2) as well as replacing uridine residues with 2' deoxy thymidine (*id.* 21:1-2; 40:19-29).

[24] Referring to Figure 14, Tuschl II states that substitution of the 2-nt 3' overhangs by 2'-deoxy nucleotides (d) had no effect on RNAi activity and replacement of two additional ribonucleotides adjacent to the overhangs in the paired region left significant RNAi activity. However, complete substitution of one or both siRNA strands by 2'-

deoxy residues or by 2'-O-methyl residues abolished RNAi. (Tuschl II 46:5-14).

[25] Figure 4 of Tuschl II depicts the effect of substituting the 2'-hydroxyl groups of the siRNA ribose residues. The 2'-hydroxyl groups (OH) in the strands of siRNA duplexes were replaced by 2'-deoxy (d) or 2'-O-methyl (Me). 2-nt and 4-nt 2'-deoxy substitutions at the 3'-ends are indicated as 2-nt d and 4-nt d, respectively. Uridine residues were replaced by 2'-deoxy thymidine. [Tuschl II 20:28-21:2.] Figure 4 is reproduced below.



{Figure 14 of Tuschl II depicts the effect of substituting the 2'-OH groups of siRNA ribose residues.}

[26] Agrawal discloses antisense oligonucleotides that have increased resistance to nucleases, noting that even nuclease resistant 3' capped oligonucleotides can become degraded eventually as their 3' capped end is slowly digested by a combination of endonuclease and exonuclease activities (Agrawal 1:8-9; 3:4-26).

[27] In a preferred embodiment, Agrawal discloses oligonucleotides comprising a target hybridizing region and a self-complementary

region, wherein the self-complementary region is connected to the target hybridizing region by a non-nucleic acid linker, e.g., an (ethylene glycol)$_{1-6}$ linker (Agrawal 8:22-24; 15:31-36).

[28] Agrawal further discloses an embodiment wherein the oligonucleotide is hyperstabilized by incorporating one or more 2'-O-Me ribonucleotides into the self-complementary region and using DNA within the complementary portion of the target hybridizing region or vice versa because DNA:RNA interactions are more stable than DNA:DNA interactions (Agrawal 16:24-35).

[29] Matulic-Adamic discloses incorporating chemical modifications at the 5' and/or 3' ends of ribozymes, i.e., RNAs which can enzymatically cleave other RNA molecules in a nucleotide base specific manner, to protect the ends from exonuclease degradation (Matulic-Adamic 1:14-17; 2:44-54).

C. IKK-γ: its sequence and relationship to NF-κB activation

[30] IKK-γ is also known as NEMO (NF-κB Essential Modulator) (828 patent specification 8:31-32; Kenwrick 2:39-42; Krappmann abstract).

[31] NF-κB plays a pivotal role in many cellular processes, including inflammation, the immune response, cell proliferation, and apoptosis (Krappmann 29779 ¶1).

[32] According to Krappmann, specific down regulation of IKK-γ protein levels by antisense oligonucleotides significantly reduced cytokine mediated activation of the IKK complex and subsequent NF-κB activation (Krappmann abstract; 29780, col. 2, ¶2).

[33] Kenwrick also discloses that the absence of IKK-γ protein results in a complete inhibition of NF-κB activation (Kenwrick 2:42-46).

[34] Yamaoka cloned a 48 kDa murine IKK-γ/NEMO protein and also found that it was essential for NF-κB activation (Yamaoka abstract; 1233-1234 "Molecular Cloning of NEMO;" 1238, col. 2, ¶2).

[35] Yamaoka deposited the NEMO cDNA sequence with GenBank under accession number AF069542 and provided the amino acid sequence of the protein in Figure 3 (Yamaoka 1234, 1240).

[36] We find that it is well within ordinary skill in the art to access the publicly available gene sequence database GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html) to obtain the NEMO cDNA sequence reported by Yamaoka under accession number AF069542.

[37] Smahi discloses that most cases of familial incontinentia pigmenta (IP), a genodermatosis that is usually lethal prenatally in males and that causes abnormalities of the skin, hair, nails, teeth, eyes, central nervous system in affected females, are caused by mutations in the NEMO/IKK-γ gene (Smahi abstract).

[38] Kenwrick discloses a method treating NF-κB related medical conditions, such as IP, by administering therapeutically effective amounts of IKK-γ/NEMO nucleic acid of SEQ ID NO:1, a 23106 nt DNA (Kenwrick 4:24-28, 42-43; 49: <210> - <213>).

[39] Kenwrick also discloses approximately sixty-seven sequences, generally about 20 nt in length, for use as primers and probes in

assays and kits for detecting mutations of SEQ ID NO:1 in an organism (Kenwrick 5:33-7:29; SEQUENCE LISTING, cols. 73-90).

III. Discussion

As noted above, the pending rejections fall into two groups. While the primary references (i.e., Tuschl I, Ebashir (alone or in combination with Bass), or Fire) in each rejection of the first group are different, each rejection relies on common secondary (i.e., Tuschl II, Yamaoka, Krappmann, and Smahi) and tertiary (i.e., Agrawal and Matulic-Adamic) references, thereby raising common issues. Consequently, these rejections are addressed together.

A. The first group of rejections

1. Findings and conclusions of the Examiner

a. claim 1

As to claim 1, the Examiner found that Tuschl I teaches using 21-23 nt dsRNA molecules with 2'-deoxy modifications to mediate RNAi, if the sequence of the gene to be targeted is known, because siRNAs are effective at concentrations several orders of magnitude below concentrations used in conventional antisense or ribosome gene targeting experiments (Ans. 6). The Examiner found that Tuschl I differs in failing to disclose siRNA molecules having both 2'-O-methyl and 2'-fluoro modifications (Ans. 6) and that down regulate the IKK-γ gene (Ans. 7).

The Examiner found that Elbashir teaches that 21 nt siRNAs with 2 nt 3' overhangs and 2'-thymidine modifications mediate RNAi at concentrations several orders of magnitude lower than concentrations used in conventional antisense or ribosome gene targeting experiments (Ans. 12).

The Examiner found that Bass teaches that once the sequence of gene is known, RNAi may be used to determine its function, that RNAi is a routine technology, and that siRNA mediated RNAi triggers degradation of mRNA at concentrations several orders of magnitude lower than concentrations used in conventional antisense or ribosome gene targeting experiments (Ans. 13). The Examiner found that Elbashir differs in failing to disclose siRNA molecules having both 2'-O-methyl and 2'-fluoro modifications (Ans. 12) and that neither Elbashir nor Bass disclose siRNAs that down regulate the IKK-γ gene (Ans. 13).

The Examiner found that Fire discloses dsRNA mediated inhibition of gene expression, wherein the dsRNA comprised (i) at least 25 base pairs, (ii) complementary sense and antisense strands, the antisense strand being at least 90% complementary to the nucleotide sequence of a target gene, and (iii) optionally, one or more modifications to either the phosphate-sugar backbone or nucleoside (Ans. 16). The Examiner found that Fire discloses that dsRNA mediated RNAi is at least 100-fold more effective than an equivalent concentration of antisense RNA in reducing gene expression (Ans. 17). The Examiner found that Fire differs in failing to disclose siRNA molecules having both 2'-O-methyl and 2'-fluoro modifications and that down regulate the IKK-γ gene (Ans. 17).

The Examiner found that Tuschl II teaches 19-25 nt dsRNAs with 3' end overhangs which act as guide RNAs for sequence specific mRNA degradation and show high stability *in vivo* or *in vitro* (Ans. 6, 12, 1). The Examiner also found that Tuschl II teaches substituting 2' OR (wherein R is

C_{1-6} alkyl) and/or fluoro groups for the 2' OH groups of the RNA sugars (Ans. 6-7, 13, 17).

The Examiner found that Yamaoka discloses the cDNA sequence encoding a 48 kDa IKK-γ protein (Ans. 7, 13, 17).

The Examiner found that Krappmann discloses that specific down regulation of IKK-γ protein levels by antisense oligonucleotide significantly reduced cytokine mediated activation of IKK complex and subsequent NF-κB activation (Ans. 7, 13, 18).

The Examiner found that Smahi identifies the IKK-γ gene as target for experimental gene silencing because mutations in this gene are associated with the genetic disease IP (Ans. 7, 14, 18).

The Examiner found that the level of skill in the art is high (Ans. 8, 14, 18).

The Examiner concluded that it would have been obvious to combine the teachings of Tuschl I or Elbashir or Fire with the teachings of Tuschl II to produce chemically modified, stable ds siRNA (Ans. 7, 13, 17). The Examiner further concluded that it would have been obvious to combine the teachings of Tuschl I or Elabshir or Fire with the teachings of Tuschl II and with the teachings of Smahi, Krappmann, and Yamaoka to produce siRNA molecules that down regulate the IKK-γ gene because Krappmann discloses antisense mediated down regulation of the IKK-γ gene, Smahi teaches that the IKK-γ gene is a desirable target, and Tuschl I, Elbashir, and Fire each teach that siRNAs are more powerful than conventional antisense or ribozyme mediated gene targeting experiments (Ans. 7-8, 14, 18). The Examiner concluded that one of ordinary skill in the art would have had a

reasonable expectation of success of producing chemically modified siRNA molecules that down regulate the IKK-γ given the teachings of the applied references and the high level of skill in the art.

b. claim 4

As to claim 4, the Examiner found that none of Tuschl I, Elbashir, Bass, Fire, Tuschl II, Smahi, Krappmann or Yamaoka discloses connecting the sense and antisense strands of the siRNA by a linker (Ans. 8, 14, 18).

The Examiner found that Agrawal discloses antisense oligonucleotides with increased resistance to degradation by nucleases, including both exo- and endo-nucleases; and, which formed stable hybrids with target sequences and activated RNase H (i.e., mediated RNAi) (Ans. 8, 14, 19). The Examiner found that the antisense oligonucleotide of Agrawal contained (i) a self-complementary region which self-paired, resulting in formation of a hairpin loop containing a polynucleotide linker; and, (ii) a target hybridization region which may be connected to the self-complementary region via a non-nucleic acid linker (Ans. 8, 15, 19).

The Examiner concluded that it would have been obvious to combine the teachings of Agrawal with the teachings of the references applied against claim 1 because Agrawal suggests forming stable, nuclease-resistant duplexes using linkers capable of mediating RNAi (Ans. 8, 15, 19).

c. claim 7

As to claim 7, the Examiner found that none of Tuschl I, Elbashir, Bass, Fire, Tuschl II, Smahi, Krappmann or Yamaoka discloses fragments [sic, overhangs?] comprising terminal caps (Ans. 9, 15, 19).

The Examiner found that Matulic-Adamic discloses chemically capping the 5' and/or 3' ends of ribozymes, i.e., nucleic acids that are particularly use for enzymatic cleavage of RNA, to protect the ribozyme from exonuclease degradation (Ans. 9, 15, 19).

The Examiner concluded that it would have been obvious to combine the teachings of Matulic-Adamic with the teachings of the references applied against claim 1, i.e., to end cap the siRNA, because Matulic-Adamic teaches that end capping protects against exonuclease degradation (Ans. 9, 15, 19-20).

2. Appellant's arguments

Initially, we note that arguments based on evidence which has not been cited as relied upon in the Evidence Appendix of the principal brief or provided therewith have not been considered. 37 C.F.R. § 41.37(c)(2)(ix).

a. claim 1

In essence, Appellant argues that there was little, if any, reason for one of ordinary skill in the art to modify siRNAs with both 2'-O-methyl and 2'-deoxy-2'-fluoro modifications because one could not have predicted which modifications might be efficacious and because Tuschl II was said to teach away for the claimed modification (App. Br. 12-13).

Specifically Appellant argues that Tuschl II expressly warns against using more than two 2'-deoxy modified nucleotides at the strands' 3'-ends or using any 2'-O-methyl modifications, i.e.:

> 2'-deoxy substitutions of the 2-nt 3' overhanging ribonucleotides do not affect RNAi, but help to reduce the costs of RNA synthesis and may enhance RNAse resistance of siRNA duplexes. More extensive 2'-deoxy or 2'-O-methyl

> modifications, however, reduce the ability of siRNAs to mediate RNAi, probably by interfering with protein association for siRNAP assembly. [Tuschl II 49:28-50:2.]

(App. Br. 13). Appellant further argues that since Tuschl II fails to state expressly the effect of 2'-O-methyl substitution on page 46, Tuschl II can only be read teaching the entire avoidance of 2'-O-methyl modifications (App. Br. 13). Thus, Appellant concludes:

> [b]ecause 2'-deoxy and 2'-O-methyl modifications were regarded as beneficial to other nucleic acid inhibitors known in the art at the time, and these modifications are found by Tuschl to be not tolerated at all (in the case of 2'-O-methyl modification), or under very limited circumstances (in the case of 2'-deoxy modification), the art of chemically modifying siRNA molecules was highly unpredictable, and the ... ribozyme and/or antisense art predating the present invention does nothing to alleviate this unpredictability. [App. Br. 14.]

Appellant further argues that since Smahi shows that mutation of the IKK-γ gene result in death or disease, one skilled in the art would not have been motivated to down regulate the IKK-γ gene unless he was interested in causing death or disease (App. Br. 14-15). Appellant points out that Elbashir fails to disclose an siRNA that down regulates IKK-γ gene (App. Br. 19).

Appellant also argues that Yamaoka does not disclose an IKK-γ cDNA sequence (App. Br. 15).

Appellant argues that Bass teaches that RNAi can occur by different pathways, one mediated by long dsRNA (at least 30 nt in length) molecules

and the other by short dsRNA (siRNA) molecules; and, that Bass' reference to RNAi as a routine laboratory procedure refers to the long dsRNA pathway (App. Br. 19, 21). Thus, Appellant reasons, one of ordinary skill in the art would have understood Fire to have mistakenly stated that dsRNA molecules of 25 nt in length were operative because dsRNA molecules smaller than 30 nts were inoperative (App. Br. 21).

b. claim 4

Appellant argues that antisense molecules are substantially single stranded prior to interacting with their target and, thus, more susceptible to nuclease attack than is a double stranded nucleic acid molecule (App. Br. 16). Moreover, when Tuschl II limited applicability of 2'-deoxy modifications and avoidance of 2'-O-methyl modifications are considered, Agrawal does not inform or lend predictability to the art of chemically modified siRNA molecules (App. Br. 16). [See also App. Br. 20, 22.]

c. claim 7

In a similar vein, Appellant argues that ribozymes are substantially single stranded prior to interacting with their target and, thus, more susceptible to nuclease attack than is a double stranded nucleic acid molecule (App. Br. 17). Moreover, when Tuschl II limited applicability of 2'-deoxy modifications and avoidance of 2'-O-methyl modifications are considered, Matulic-Adamic does not inform or lend predictability to the art of chemically modified siRNA molecules (App. Br. 17-18). [See also App. Br. 20, 22.]

3. Legal principles

"Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406, 127 S. Ct. 1727 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art, and (4) where in evidence, so-called secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). In *KSR*, the Supreme Court reaffirmed principles based on its precedent that "[t]he combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." *KSR,* 550 U.S. at 416. The Court emphasized that "rejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness" *id.* at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)). However, "the analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *Id.* Thus, "[i]n considering motivation in an obviousness analysis, we ask 'whether a person of ordinary skill in the art, possessed with the understandings and knowledge reflected in the prior art,

and motivated by the general problem facing the inventor, would have been led to make the combination recited in the claims.'" *Optivus Tech., Inc. v. Ion Beam Applications S.A.*, 469 F.3d 978, 990 (Fed. Cir. 2006) (citing *In re Kahn*, 441 F.3d at 988). "Non-obviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references." *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097 (Fed. Cir. 1986).

4. Analysis

Here, Tuschl I, Elbashir, and Fire each teach dsRNAs about 18 to about 28 nucleotides in length (FF 7, 10, 13, 15, 16) that mediate RNAi (FF 3, 4, 7-8, 9-10, 12, 15) at concentrations several orders of magnitude below the concentrations needed in conventional antisense or ribozyme gene targeting experiments and, thus, suggest the usefulness of these siRNAs in gene targeting experiments (FF 5, 6). Gene targeting may be used to inhibit expression of a gene associated which encodes a protein associated with or causative of disease or other undesirable conditions or to study the function of gene (FF 2, 8). Tuschl II discloses siRNA duplexes 19-25 nucleotides in length that mediate RNAi (FF 19) and a proposed molecular explanation of how siRNA mediated RNAi works (FF 19).

Tuschl I and Tuschl II teach and/or suggest that the most effective siRNA molecules comprise two 21 nt strands which are base paired such that 1-3, especially 2 nt 3' overhangs are present on both ends of the siRNA duplex (FF 13, 18). Thus, each strand of the siRNA is single-stranded in its 3' overhang. According to both Tuschl I and Tuschl II, the absence of a 2'-OH group enhances the nuclease resistance of the 3' overhang (FF 14, 22).

Moreover, Fire, Tuschl I, Elbashir, and Tuschl II each teach the siRNA may include modifications to phosphate-sugar backbone and/or nucleoside (FF 7, 13, 16, 21).

Modifications of nucleic acids to stabilize them against degradation by nucleases was known in the art. Tuschl II preferably substitutes the 2'-OH groups of 3' overhang (i.e., single stranded) portion of siRNAs with OR groups to form C_{1-6} alkyl ester groups, e.g., methyl esters, and halide groups, e.g., fluoro, as well as replacing uridine residues with 2'-deoxy thymidines, to enhance the nuclease resistance of the overhang portion (FF 23). Similarly, Matulic-Adamic chemically modifies the 5' and/or 3' ends of ribozymes to protect them from exonuclease degradation (FF 29). Agrawal notes that even nuclease resistant 3' capped oligonucleotides can become degraded eventually as their 3' capped end is slowly digested by a combination of endonuclease and exonuclease activity (FF 26) and discloses antisense oligonucleotides wherein regions of the antisense are connected via linkers (FF 27).

Therefore, we agree with the Examiner that it would have been obvious to modify an siRNA, particularly at nucleotides in a 1-3 nt of a 3' overhang at the end of one or both strands, to increase the resistance of siRNA to nuclease degradation using modifications known and used for increasing the stability of RNA to nuclease degradation. We also agree with the Examiner that it would have been obvious to select an IKK-γ as a gene for targeted experimentation because of its known relationship with NF-κB activation as disclosed by Krappmann (FF 32) and/or its causal relationship

to IP as disclosed by Smahi (37) given Yamaoka's disclosed cDNA and amino acid sequences for an IKK-γ gene (FF 34-36).

Appellant's arguments do not persuade us to the contrary.

Nucleic acid molecules are known to be degraded or hydrolyzed by nucleases *in vivo* and in culture systems and, thus, it is routine in the art to modify nucleic acids to resist nuclease hydrolysis (see e.g., FF 16, 21, 26, 29). Exonucleases by definition hydrolyze nucleic acid molecules from their ends, while endonucleases attack nucleic molecules between their ends. Modifications which interfere with the interaction between a nucleotide substrate and a nuclease enzyme's active site would reasonably be expected to increase a nucleic acid's resistance to nuclease degradation. Thus, it is not unexpected, and in fact is entirely predictable, that a double stranded nucleic acid would be less susceptible to nuclease degradation than a single stranded nucleic acid since one strand would reasonably be expected to block a nuclease's, particularly an endonuclease's, access to the other strand to some degree. Indeed, Appellant even points out that single stranded nucleic acids are more susceptible to nuclease degradation than double stranded nucleic acids (see e.g., App. Br. 16-17). Similarly, capping one or both ends of a nucleic acid molecule, as disclosed by Matulic-Adamic, or forming a double-stranded portion within an antisense molecule, as disclosed by Agrawal, would also reasonably be expected to sterically interfere with the active site of a nuclease. By the same token, the most effective siRNA molecules are disclosed as having 3' overhangs (see e.g., FF 13, 18) and, therefore, would reasonably be expected to be more susceptible to nuclease degradation by virtue of being single stranded structures. Moreover, Tuschl

I and Tuschl II identify a specific site of nuclease attack, i.e., the 2'-OH group of the nucleotide (FF 14, 22). Therefore, it would have been obvious to modify the 2'-OH group of the 3' overhang of siRNAs to increase the nuclease resistance of the siRNA. The combination of chemical modifications known to increase the nuclease resistance of nucleic acid molecules to siRNA molecules, particularly to the 2'-OH group of a single stranded 3' overhang, is likely to be obvious when it does no more than yield a predictable result.

Furthermore, claim 1 only requires the recited siRNA to have a single 2'-O-methyl modification and a single 2'-fluoro modification. For example, if the siRNA had a 3 nt 3' overhang, the end nucleotide might be modified with a 2'-O-methyl group and the middle nucleotide of the overhang might be modified with a 2'-fluoro group. However, overmodification of the 2'-OH groups is expressly warned against by Tuschl II, e.g., complete substitution of the 2'-OH groups of one or both siRNA strands abolishes RNAi (FF 24). Moreover, Tuschl II teaches that the 3' overhang does not contribute to specificity of the target recognition (FF 19), a teaching which have reasonably suggested that chemical modifications to the 3' overhang would be less likely to interfere with the efficacy of the siRNA. Indeed, this appears to be consistent with the data presented in the bar graph of Tuschl II Figure 4 (FF 25). Thus, even assuming *arguendo* that Tuschl II warned against modifying more than two 2'-OH groups at the 3' ends of the siRNA, this argument is not convincing because modifying just two 2'-OH groups at the 3' overhangs (ends) of the siRNA is within the scope of the claimed invention.

Appellant's argument that Tuschl II teaches avoiding any 2'-O-methyl modifications (App. Br. 13) is likewise unpersuasive and misstates Tuschl II. A fair reading of Tuschl II at column 49, line 28, through column 50, line 2, as pointed out by the Examiner (see e.g., Ans. 22), is that more extensive 2'-deoxy or 2'-O-methyl modifications beyond the two nucleotide 3' overhang reduces the ability of siRNAs to mediate RNAi. Moreover, stating that *complete* substitution of one or both siRNA stands by 2'-O-methyl groups abolished RNAi (FF 24), is not synonymous with stating that *any* 2'-O-methyl group modification should be avoided. Therefore, this argument is not persuasive.

Appellant's argument that Smahi fails to provide a motivation for targeting an IKK-γ gene for down regulation by RNAi because down regulated an already defective gene could cause death or disease is unpersuasive for a number of reasons. The Examiner is relying on the combination of Krappmann and Smahi as a reason to study a down regulated IKK-γ gene. As noted by the Examiner (Ans. 25), Krappmann discloses the importance of down regulating an IKK-γ gene, i.e., down regulating IKK-γ reduces subsequent NK-κB activation, which plays a pivotal response in cellular processes, e.g., inflammation (FF 31-32). Thus, the Examiner essentially concluded that it would have been obvious to down regulate or knock down an IKK-γ gene to study the effect on cellular processes, e.g., inflammation. Smahi discloses that most cases of IP are caused by mutations in the IKK-γ gene (FF 37). The Examiner again found that the IKK-γ gene is a desirable gene for experimental gene silencing (Ans. 7, 14, 18). Thus, the Examiner evidently concluded that it would have been

obvious to experimentally silence an IKK-γ gene to provide a knock-down experimental IP study system. Therefore, Krappmann and/or Smahi suggest studying the IKK-γ gene by experimental silencing. Moreover, the claimed invention is not directed to treating any specific disease or condition by administering a therapeutically effective amount of an IKK-γ gene down regulating siRNA. Therefore, this argument is not persuasive.

Next, Appellant is correct that Yamaoka did not expressly recite the cDNA sequence he obtained. However, Yamaoka expressly stated that the cDNA sequence was deposited with GenBank under accession number AF069542 (FF 35), which is analogous to a deposit of biological material made to satisfy the written description requirement of 35 U.S.C. § 112, first paragraph. It would have been well within ordinary skill in the art, which as the Examiner noted is high, to obtain the deposited cDNA sequence from the publicly available GenBank database (FF36). This is entirely routine and predictable (FF36). Alternatively, Yamaoka expressly disclosed the amino acid sequence of the protein encoded by the cDNA (FF 35), thereby providing a generic description of the DNA sequences encoding the IKK-γ protein. *See e.g., In re Wallach*, 378 F.3d 1330, 1333-34 (Fed. Cir. 2004) (an amino acid sequence supports the entire genus of DNA sequences that can encode the amino acid sequence because the state of the art has developed such that it is routine matter to convert one to the other). Therefore, Appellant's argument that Yamaoka did not disclose an IKK-γ cDNA sequence is not well taken.

Finally, Appellant's argument that one of ordinary skill in the art would interpret Fire as inoperative for dsRNAs of 25 nucleotides in length is

not persuasive. A patent is presumed valid. 35 U.S.C. § 282. In addition, claim 10 of Fire expressly recites a method of RNAi wherein the dsRNA is at least 25 bases in length (FF 7). "The burden of establishing invalidity of a patent or any claim thereof shall rest on the party asserting such invalidity." 35 U.S.C. § 282. Appellant's conclusory statement that one of ordinary skill in the art would have understood Fire to be mistaken in its specification and claims is insufficient to meet this burden. *In re Schulze*, 346 F.2d 600, 602 (CCPA 1965) (argument in the brief does not take the place of evidence of record).

Based on the foregoing, we sustain the rejections of

claims 1-3, 14, and 16 under § 103 over the combined teachings of Tuschl I, Tuschl II, Yamaoka, Smahi, and Krappmann; claims 4-6 under § 103 over the combined teachings of Tuschl I, Tuschl II, Yamaoka, Smahi, Krappmann, and Agrawal; claims 7 and 8 under § 103 over the combined teachings of Tuschl I, Tuschl II, Yamaoka, Smahi, Krappmann, and Matulic-Adamic;

claims 1-3 and 14-16 under § 103 over the combined teachings of Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, and Smahi; claims 4-6 under § 103 over the combined teachings of Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, Smahi, and Agrawal; claims 7-8 under § 103 over the combined teachings of Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, Smahi, and Matulic-Adamic;

claims 1-3, 9, and 14-16 under § 103 over the combined teachings of Fire, Tuschl II, Yamaoka, Krappmann, and Smahi; (I) claims 4-6 under § 103 over the combined teachings of Fire, Tuschl II, Yamaoka, Krappmann,

Smahi, and Agrawal; and, claims 7-8 under § 103 over the combined teachings of Fire, Tuschl II, Yamaoka, Krappmann, Smahi, and Matulic-Adamic.

B. The second group of rejections

The second group of rejections only contains a single rejection, i.e., claims 1-3 are rejected under § 103 over the combined teachings of Kenwrick, Tuschl I, and Tuschl II.

1. Findings and conclusions of the Examiner

The Examiner found that Kenwrick teaches methods of treating NK-κB related medical conditions by administering therapeutically effective amounts of SEQ ID NO:1 (Ans. 10, 32). Kenwrick SEQ ID NO:1 is a 23106 nt IKK-γ DNA sequence (FF 38). The Examiner also found that Kenwrick discloses "a large number of short nucleic acids," five of which are 100% complementary to an IKK-γ mRNA (SEQ ID NOs:49, 50, 54, 56, and 61) (Ans. 10). The Examiner found that Kenwrick differs in failing to disclose siRNA molecules comprising 2'-O-methyl and 2'-fluoro group modifications, wherein the siRNA molecules also down regulate expression of the IKK-γ gene (Ans. 10).

The Examiner concluded that it would have been obvious to produce double stranded siRNA molecules that down regulate IKK-γ gene expression since Kenwrick teaches IKK-γ gene sequences and Tusch I and Tuschl II teach methods of producing chemically modified double stranded siRNA molecules (Ans. 10-11, 32-33).

2. Appellant's arguments

Appellant argues that Kenwrick teaches DNA fragments which are used as primers to amplify and detect IKK-γ nucleic acids and that Kenwrick does not teach down regulation of IKK-γ gene expression for any reason. In essence, Appellant argues the rejection of claims 1-3 over Kenwrick, Tuschl I, and Tuschl II is a classic hindsight reconstruction of the claimed invention.

3. Legal principles

"One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988).

4. Analysis

Here, we agree with Appellant. Kenwrick discloses a method of treating NK-κB related medical conditions by administering therapeutically effective amounts of the IKK-γ gene, i.e., SEQ ID NO:1 (FF 38). Implicitly, Kenwrick is seeking to correct an abnormal IKK-γ gene. This interpretation is consistent with Kenwrick's disclosure of well over sixty short DNA sequences as primers and probes in assays and kits for detecting mutations in SEQ ID NO:1, i.e., the IKK-γ gene (FF 39). The Examiner has failed to explain why Kenwrick would have suggested down-regulating a gene that he is trying to repair, i.e., replace with SEQ ID NO:1. Rather, it appears that the Examiner has fallen into the trap of hindsight reconstruction.

Based on the foregoing, we reverse the rejection of claims 1-3 under § 103 over the combined teachings of Kenwrick, Tuschl I, and Tuschl II.

D. Conclusion

Appellant has failed to show the Examiner reversibly erred in (i) finding that Yamaoka disclosed the cDNA sequence of an IKK-γ gene, (ii) concluding that an ordinarily skilled artisan would have had both a motivation to and a reasonable expectation of success of providing an siRNA molecule with at least one 2'-O-methyl group and at least one 2'-fluoro group, (iii) failing to provide a reason for down regulating an IKK-γ gene, (iv) concluding that it would have been obvious to join a sense and an anti-sense RNA strand into a single ds siRNA via a linker molecule based on the teachings of Agrawal, or (v) concluding that it would have been obvious to cap either or both ends of the antisense strand based on the teachings of Matulic-Adamic. However, Appellant has shown that the Examiner reversibly erred in (vi) concluding that Kenwrick provided a teaching or suggestion of down regulating IKK-γ gene expression.

IV. Order

Upon consideration of the record, and for the reasons given, it is

ORDERED that the decision of the Examiner to reject claims 1-3, 14, and 16 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I and Tuschl II in view of Yamaoka, Smahi, and Krappmann is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I, Tuschl II, Yamaoka, Smahi, and Krappmann taken further in view of Agrawal is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 7 and 8 under 35 U.S.C. § 103(a) as unpatentable over Tuschl I,

Tuschl II, Yamaoka, Smahi, and Krappmann taken further in view of Matulic-Adamic is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 1-3 under 35 U.S.C. § 103(a) as unpatentable over Kenwrick in view of Tuschl I and Tuschl II is REVERSED;

FURTHER ORDERED that the decision of the Examiner to reject claims 1-3 and 14-16 under 35 U.S.C. § 103(a) as unpatentable over Elbashir, Tuschl II, and Bass in view of Yamaoka, Krappmann, and Smahi is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, and Smahi further in view of Agrawal is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 7-8 under 35 U.S.C. § 103(a) as unpatentable over Elbashir, Tuschl II, Bass, Yamaoka, Krappmann, and Smahi further in view of Matulic-Adamic is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 1-3, 9, and 14-16 under 35 U.S.C. § 103(a) as unpatentable over Fire and Tuschl II in view of Yamaoka, Krappmann, and Smahi is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 4-6 under 35 U.S.C. § 103(a) as unpatentable over Fire, Tuschl II, Yamaoka, Krappmann, and Smahi further in view of Agrawal is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner to reject claims 7-8 under 35 U.S.C. § 103(a) as unpatentable over Fire, Tuschl II, Yamaoka, Krappmann, and Smahi further in view of Matulic-Adamic is AFFIRMED; and,

FURTHER ORDERED that requests for extending time for taking any subsequent action in connection with this appeal are governed by 37 C.F.R. § 1.550(c).

AFFIRMED

Ack

Enc: [1] Lehninger PRINCIPLES OF BIOCHEMISTRY, fourth edition, W.H. Freeeman and Company, New York (2005), pp. 921-922.

cc:

Sirna Therapeutics, Inc.
1700 Owens Street
4th Floor
San Francisco CA 94158

Third Party Requestor:

Scott E. Kamholz
Foley Hoag, LLP
155 Seaport Road
Boston, MA 02210-2600

Lehninger

PRINCIPLES OF BIOCHEMISTRY

fourth edition

DAVID L. NELSON
Professor of Biochemistry
University of Wisconsin–Madison

MICHAEL M. COX
Professor of Biochemistry
University of Wisconsin–Madison

W. H. Freeman and Company
New York

Publisher: Sara Tenney
Acquisitions Editor: Katherine Ahr
Development Editor: Morgan Ryan
Marketing Manager: Sarah Martin
Marketing Director: John Britch
Project Editor: .Jane O'Neill
Design Manager: Blake Logan
Text Designer: Rae Grant
Cover Designer: Yuichiro Nishizawa
Page Makeup: Paul Lacy
Illustration Coordinator: Shawn Churchman
Illustrations: Fine Line Illustrations
Molecular Graphics/Cover Illustration: .Jean-Yves Sgro
Photo Editor: Vikii Wong
Production Coordinator: Paul Rohloff
Media & Supplements Editors: Jeffrey Ciprioni, Melanie Mays, Nick Tymoczko
Media Developers: Sumanas, Inc.
Composition: TechBooks
Manufacturing: RR Donnelley & Sons Company

On the cover: The F_1 ATPase, part of a complex responsible for ATP synthesis in eukaryotic mitochondria. See Chapter 19.

Library of Congress Control Number: 2004101716

ISBN: 0-7167-4339-6
EAN: 97807167743392

Printed in the United States of America

Fourth printing

W. H. Freeman and Company
41 Madison Avenue
New York, NY 10010
Houndmills, Basingstoke RG21 6XS, England

www.whfreeman.com

PART III

INFORMATION PATHWAYS

The third and final part of this book explores the biochemical mechanisms underlying the apparently contradictory requirements for both genetic continuity and evolution of living organisms. What is the molecular nature of genetic material? How is genetic information transmitted from one generation to the next with high fidelity? How do the rare changes in genetic material that are the raw material of evolution arise? How is genetic information ultimately expressed in the amino acid sequences of the astonishing variety of protein molecules in a living cell?

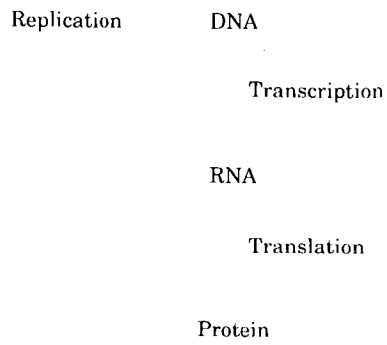
The fundamental unit of information in living systems is the **gene.** A gene can be defined biochemically as a segment of DNA (or, in a few cases, RNA) that encodes the information required to produce a functional biological product. The final product is usually a protein, so much of the material in Part III concerns genes that encode proteins. A functional gene product might also be one of several classes of RNA molecules. The storage, maintenance, and metabolism of these informational units form the focal points of our discussion in Part III.

Modern biochemical research on gene structure and function has brought to biology a revolution comparable to that stimulated by the publication of Darwin's theory on the origin of species nearly 150 years ago. An understanding of how information is stored and used in cells has brought penetrating new insights to some of the most fundamental questions about cellular structure and function. A comprehensive conceptual framework for biochemistry is now unfolding.

Today's understanding of information pathways has arisen from the convergence of genetics, physics, and chemistry in modern biochemistry. This was epitomized by the discovery of the double-helical structure of DNA, postulated by James Watson and Francis Crick in 1953 (see Fig. 8–15). Genetic theory contributed the concept of coding by genes. Physics permitted the determination of molecular structure by x-ray diffraction analysis. Chemistry revealed the composition of DNA. The profound impact of the Watson-Crick hypothesis arose from its ability to account for a wide range of observations derived from studies in these diverse disciplines.

This revolution in our understanding of the structure of DNA inevitably stimulated questions about its function. The double-helical structure itself clearly suggested how DNA might be copied so that the information it contains can be transmitted from one generation to the next. Clarification of how the information in DNA is converted into functional proteins came with the discovery of both messenger RNA and transfer RNA and with the deciphering of the genetic code.

These and other major advances gave rise to the central dogma of molecular biology, comprising the three major processes in the cellular utilization of genetic information. The first is **replication,** the copying of parental DNA to form daughter DNA molecules with identical nucleotide sequences. The second is **transcription,** the process by which parts of the genetic message encoded in DNA are copied precisely into RNA. The third is **translation,** whereby the genetic message encoded in messenger RNA is translated on the ribosomes into a polypeptide with a particular sequence of amino acids.



The central dogma of molecular biology, showing the general pathways of information flow via replication, transcription, and translation. The term "dogma" is a misnomer. Introduced by Francis Crick at a time when little evidence supported these ideas, the dogma has become a well-established principle.

Part III explores these and related processes. In Chapter 24 we examine the structure, topology, and packaging of chromosomes and genes. The processes underlying the central dogma are elaborated in Chapters 25 through 27. Finally, we turn to regulation, examining how the expression of genetic information is controlled (Chapter 28).

A major theme running through these chapters is the added complexity inherent in the biosynthesis of macromolecules that contain information. Assembling nucleic acids and proteins with particular sequences of nucleotides and amino acids represents nothing less than preserving the faithful expression of the template upon which life itself is based. We might expect the formation of phosphodiester bonds in DNA or peptide bonds in proteins to be a trivial feat for cells, given the arsenal of enzymatic and chemical tools described in Part II. However, the framework of patterns and rules established in our examination of metabolic pathways thus far must be enlarged considerably to take into account molecular information. Bonds must be formed between *particular* subunits in informational biopolymers, avoiding either the occurrence or the persistence of sequence errors. This has an enormous impact on the thermodynamics, chemistry, and enzymology of the biosynthetic processes. Formation of a peptide bond requires an energy input of only about 21 kJ/mol of bonds and can be catalyzed by relatively simple enzymes. But to synthesize a bond between two specific amino acids at a particular point in a polypeptide, the cell invests about 125 kJ/mol while making use of more than 20 enzymes, RNA molecules, and specialized proteins. The chemistry involved in peptide bond formation does not change because of this requirement, but additional processes are layered over the basic reaction to ensure that the peptide bond is formed between particular amino acids. Information is expensive.

The dynamic interaction between nucleic acids and proteins is another central theme of Part III. With the important exception of a few catalytic RNA molecules (discussed in Chapters 26 and 27), the processes that make up the pathways of cellular information flow are catalyzed and regulated by proteins. An understanding of these enzymes and other proteins can have practical as well as intellectual rewards, because they form the basis of recombinant DNA technology (introduced in Chapter 9).